Research Article

Use of physical blockers to control invasive red swamp crayfish in burrows

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Abstract

The red swamp crayfish *Procambarus clarkii* is native to the southeast United States but has successfully invaded nearly every continent around the world. Although physical, biological, and chemical controls are employed to reduce or eliminate populations in open-water systems, terrestrial burrows provide a potential refuge from aquatic control treatments. We conducted burrow trials to test whether two physical blocker treatments would kill *P. clarkii* in their burrows. Bentonite clay (a sealing agent) and expanding foam (an insulating sealant) were each applied to 37 crayfish burrows, and 36 burrows served as treatment controls (i.e., 110 total burrows). Burrows were excavated 48 hr after the application of the physical blockers to assess the status of crayfish in treated and control burrows. There was 74% mortality of crayfish in occupied burrows treated with bentonite clay, 62% in burrows treated with expanding foam, and 6% mortality in control burrows. We believe bentonite clay should continue to be field-tested; however, because expanding foam is toxic to aquatic organisms and is expected to persist in the environment, we do not believe it is a suitable physical blocker for the control of invasive crayfish in burrows. Bentonite clay applications likely will not need permits, will mitigate damage to banks and levees caused by burrowing crayfish, and can be used with other control agents such as pesticides. However, the use of physical blockers may be limited at field sites that have burrows with complex morphologies. We believe the use of bentonite clay to control invasive crayfish in terrestrial burrows will provide resource managers with an effective tool for their integrative pest management programs.

Key words: *Procambarus clarkii*, crayfish burrows, invasive aquatic species, physical blockers, bentonite clay

Introduction

Red swamp crayfish (*Procambarus clarkii* [Girard, 1852]) are native to the southern United States and northeastern Mexico but have established populations on every continent except Antarctica and Australia (Hobbs et al. 1989; Loureiro et al. 2015; Oficialdegui et al. 2020). Several life-history traits make it a highly invasive crayfish including their ability to tolerate a wide range of environmental temperatures (Chucholl 2011; Peruzza et al. 2015), their high fecundity (e.g., large number of eggs, multiple reproductive cycles per year) (Oluoch 1990; Huner and Barr 1991; Huner and Lindqvist 1995; Jin et al. 2019), and their ability to traverse overland from permanent wetlands (Siesa et al. 2011) to forage or seek new shelter (Gherardi and Barbaresi 2000; Gherardi et al. 2000; Thomas et al. 2019; Jussila et al. 2021). Additionally, *P. clarkii* can use aerial respiration to survive for a month or longer out of water, which aids their movement overland (Reynolds et al. 2013; Piersanti et al. 2018).

Procambarus clarkii are considered secondary or tertiary burrowers (Huner and Barr 1991; Correia and Ferreira 1995; Jurcak et al. 2016; Haubrock et al. 2019), and their burrows may be simple or complex, ranging from one tunnel and entrance to multiple branches and entrances (Huner and Barr 1991; Correia and Ferreira 1995). Their burrows can be found in swamps, marshes, the banks of reservoirs and streams (Huner and Barr 1991; Correia and Ferreira 1995; Faller et al. 2016), and in irrigation ditches or channels of rice-fields (Correia and Ferreira 1995; Barbaresi et al. 2004a, 2004b). *Procambarus clarkii* spend about half of their lives out of surface waters, retreating to their burrows to reproduce or avoid desiccation (Oluoch 1990; Huner and Barr 1991; Gherardi et al. 2002; Ilhéu et al. 2003; Souty-Grosset et al. 2014). They leave their burrows to forage or seek new shelter, showing no faithfulness to the burrows they construct after about 12 hr (Ilhéu et al. 2003). Mean density of *P. clarkii* burrows (0.013–6.28 burrows m^{-2}) may increase in soils with higher amounts of fine sediment (Correia and Ferreira 1995; Barbaresi et al. 2004a), and burrow depths may range from 0.28 m to 0.58 m (Correia and Ferreira 1995). Burrowing can reduce bank stability in riparian environments and on levees and can damage economically important rice crops (Anastácio and Marques 1997; Anastácio et al. 2005; Faller et al. 2016).

The life history and mobility of *P. clarkii* pose significant challenges for resource managers. Open-water treatments such as pesticides, carbon dioxide, and trapping have been the focus of control efforts for *P. clarkii*; however, these methods may not be effective for crayfish in burrows (Chang and Lange 1967; Cecchinelli et al. 2012; Peay et al. 2019; Fredricks et al. 2020; Abdelrahman et al. 2021). Because of this, there is continued interest in developing effective treatments for controlling *P. clarkii* in burrows.

In this study, we evaluated (1) the effectiveness of two physical blockers, bentonite clay (a pond and levee sealing agent) and expanding foam (an insulating sealant) to kill *P. clarkii* in their burrows, and (2) the physical and biological factors that may influence the effectiveness of bentonite clay and expanding foam.

Materials and methods

Study crayfish

During the spring and summer 2021, we trapped *P. clarkii* from ponds at E.W. Shell Fisheries Research Center, Auburn, AL 36830 (32°39′09.2″N; 85°29′08.9″W) using vinyl-coated Frabill traps (42 cm × 23 cm; Frabill, Plano, IL,

Figure 1. Photograph of the research ponds used in the experiment. Catch basins are the smaller rectangular structures in ponds. Photograph by JA Stoeckel, Auburn University.

USA) that were baited with canned wet cat food (9Lives seafood and poultry favorites variety pack, Big Heart Pet, Inc., Walnut Creek, CA, USA). Trapped crayfish were subsequently held in eight indoor, flow-through raceways (3.1 m × 0.8 m) receiving water from a nearby 8.1-ha supply reservoir, which was the same water source for ponds. Polyvinyl chloride (PVC) ribbon (Bio-Fill, Pentair Aquatic Eco-Systems, Inc., Apopka, FL, USA) and PVC pipe segments (10-cm lengths by 2.54–3.81-cm inner diameter) were placed in each trough to provide refuge for crayfish. Crayfish were fed *ad libitum* commercial shrimp feed pellets (SH. Grower SI-35 3/32", Ziegler Bros, Inc., Gardners, PA, USA), three times per week until the initiation of pond studies. A maximum of 200 crayfish were held in each raceway (80 individuals m^{-2}). Newly trapped crayfish were continually added to the eight raceways throughout the year as resident crayfish were removed for the experimental trials. No crayfish were used for more than one trial. Crayfish sex was determined by the presence of gonopods between the last pair of walking legs for males or the presence of a circular seminal receptacle between the last pair of walking legs for females (Huner and Barr 1991). Crayfish were evenly distributed into each pond at a sex ratio of 1:1; however, crayfish were not selected for size before being randomly distributed into research ponds (see below), which were drained to induce burrowing by the stocked crayfish.

Burrow initiation

Trials were conducted in four 0.02-ha (~ 14 m × ~ 14 m) earthen-bottom research ponds (hereafter H19, H20, H24, H25) which were comprised of ambient sand/silt/clay soils (Figure 1). Each pond was initially filled with water, leaving approximately 1–2 m of exposed shoreline between the water edge and concrete walls of the ponds. On April 1, 280 crayfish were stocked into each of the four ponds. After two weeks, we induced burrowing by lowering standpipes in each pond every 24 hr to draw the water down to create 0.5–1 m of "new" pond bottom from the previous wetted edge. We lowered standpipes to create "new" edge for three days until ponds were drained down to the edge of the catch basins of the ponds.

Due to the small number of burrows observed over the next several weeks, we re-flooded the four ponds on May 22, added 200 crayfish to each pond, and slowly drew the water down as previously described. This resulted in 26 burrows in pond H24, but few in the other ponds. We then re-flooded approximately half the surface area of each of the three ponds and added an additional 220 crayfish to each from June 20–23, after which we lowered the standpipes, slowly draining the water until it was within ~ 1 m of the catch basins. Water was maintained at that level and a total of ~ 104 additional crayfish were added to each of the four ponds; therefore, a total of ~ 800 crayfish were stocked into each pond between April 1 and September 2 prior to the initiation of our experimental trials. During this period, ponds were checked weekly and newly formed burrows were marked with PVC flags (12.7 cm × 20.3 cm with 53 cm staff; Presco, Sherman, TX, USA) and assigned a unique identifier.

Selection of physical blockers

We selected two types of physical blockers to investigate their ability to kill crayfish in burrows. We were limited to two types of physical blockers because of the small number of burrows that resulted from repeatedly draining down the four research ponds. We selected a synthetic and natural product to compare to each other and to control burrows.

Expanding foam was selected as a possible physical blocker because we previously used expanding foam (Great Stuff Pro Gaps and Cracks, Grainger, Lake Forest, IL, USA) to create casts of burrows for research and outreach activities. It was relatively inexpensive, easy to use, sets up within 24–48 hr, and reliably reached groundwater of shallow burrows. We also discovered that crayfish were frequently coated with the foam or immobilized in the water of the terminal chamber and therefore they were functionally dead at the time of excavation. Hazards associated with the use of expanding foam are that it is a flammable aerosol and skin, eye, and respiratory irritant (Great Stuff 2019). Components of expanding foam are highly mobile in soils, are expected to degrade slowly in the environment, and are toxic to aquatic organisms (Great Stuff 2019).

We selected bentonite clay (Benseal, Halliburton, Houston, TX, USA) because it was a natural sealant used to line ponds and lagoons and we thought it may repair damage caused by crayfish burrowing in banks and levees. We believed that clay particles would plug burrows and coat crayfish making it difficult for crayfish to dig through the clay mixture before it hardened.

Table 1. Total number of burrows and number of occupied burrows in each pond, treatment, and trial.

	Trial 1		Trial 2	
Treatment / pond number	Number of burrows	Number of occupied burrows	Number of burrows	Number of occupied burrows
Control				
H19	5	2	5	1
H20	5	2	3	2
H24	5	4	4	4
H25	5	1	4	3
Total	20	9	16	10
Bentonite clay				
H19	5	1	5	2
H20	5	3	4	1
H24	5	4	4	1
H25	5	2	4	1
Total	20	10	17	5
Expanding foam				
H19	5	2	5	1
H20	5	4	4	1
H24	5	3	4	2
H25	5	0	4	0
Total	20	9	17	4

The clay mixture may also suffocate crayfish by fouling their gills or removing the air space between the burrow entrance and chamber. Bentonite clay is relatively inexpensive, readily available, and commonly used to repair ponds. The primary hazard of bentonite clay is chronically inhaling clay (silica) particles (Benseal 2017). Safety precautions include wearing protective eyewear or safety glasses, avoid breathing in product dust and washing face, hands, and other exposed skin after using the product.

Experimental design

We conducted one trial on September 15 and September 23, 2021. During the first trial, five burrows were randomly assigned to each treatment (control, expanding foam, or bentonite clay) in each of the four ponds, resulting in 60 treated burrows (Table 1). During the second trial, five burrows were assigned to each treatment in Pond H19; four to bentonite clay and expanding foam and three to the control treatment in pond H20; and four to each treatment in ponds H24 and H25, resulting in 50 treated burrows. No burrow was used more than once (Table 1).

We measured the diameter of burrow entrances (openings) (mm) using calipers (SP Bel-Art, Scienceware Model H13415-0000, Wayne, NJ, USA). We applied bentonite clay into burrows by alternatively pouring 250 mL of bentonite clay and 100 mL of pond water into burrows until we reached the burrow entrance. Expanding foam was applied by placing a 35-cm long applicator nozzle of a dispensing gun directly into burrows and slowly injecting the pressurized foam sealant until it reached the burrow entrance. The time needed to apply bentonite clay and expanding foam was recorded to the nearest minute. Burrows were excavated 48 hr after treatments using shovels and spades in both trials based on data from preliminary studies which determined crayfish were not killed but only immobilized by expanding foam for treatments lasting 24 hr. We excavated each burrow to the depth of the terminal chamber, which was slightly larger in diameter than the neck of the burrow (Huner and Barr 1991). We recorded whether the physical blockers reached the groundwater in the terminal chamber. Burrows were designated as "occupied" if at least one crayfish was present in the burrow or "unoccupied" if no crayfish was present in the burrow. We measured burrow depths with a meter stick (cm) from the burrow entrance at the surface of the ground to the terminal chamber.

Crayfish were transported back to the laboratory in labeled reclosable bags to assess their status (e.g., "live" or "dead"). All live crayfish were still moving, and all dead crayfish were limp and had begun the early stages of decomposition. Some burrows contained two *P. clarkii*, but in all cases, crayfish within the burrow were either both dead or both alive. Mortality was calculated as the proportion of burrows occupied by at least one dead crayfish for each treatment. Crayfish sex was recorded, and carapace length (CL, mm) was measured from the tip of rostrum to the posterior edge of the cephalothorax using calipers.

Data analyses

All data were analyzed for normality using a Shapiro-Wilk test and for homogeneity of variances using Levene's equal variance test. Data were normally distributed for all test variables except for the CL of crayfish treated with bentonite clay and the diameter of burrow entrances of treated burrows occupied by crayfish. Statistical significance was set at $p < 0.05$, and all data are presented as the mean ± 1 standard error. All statistical analyses were performed with R software (R Core Team 2021).

Mean CL of crayfish, burrow depth, and diameter of burrow entrances were calculated for occupied burrows treated with bentonite clay and expanding foam. For each treatment, data from occupied burrows in both trials and all ponds were pooled. Combining trials and ponds did not likely result in any bias; the main effect would be an increased error term due to unaccounted variance. For within-treatment comparisons, we used a T-test to test for significant differences in the CL between live and dead crayfish found in burrows treated with expanding foam. We used a Mann-Whitney rank sum test with continuity correction (U) to test for differences in CL between live and dead crayfish found in burrows treated with bentonite clay. We used T-tests to test for significant differences in the depth of burrows occupied by live and dead crayfish for the expanding foam treatment and the bentonite clay treatment. We also used a Mann-Whitney rank sum test with continuity correction (U) to test for differences in entrance diameter of burrows occupied by live and dead crayfish for the expanding foam treatment and the bentonite clay treatment. No comparisons of CL, burrow diameter or burrow depth between live and dead crayfish within the control treatment were made because only a single dead crayfish was found within control burrows.

We used T-tests to test for significant differences in the time it took to apply bentonite clay and expanding foam treatments. Mean application time for a given treatment was calculated for each pond x trial combination (16 total combinations: 8 combinations per each treatment). All burrows treated with either bentonite clay or expanding foam, regardless of occupancy, were included in this analysis.

We calculated the proportion of occupied burrows for each treatment x pond x trial combination (24 total combinations: 8 combinations per each of three treatments, 12 combinations per each of 2 trials) and used a two-way analysis-of-variance (ANOVA) to test whether there was a significant difference in burrow occupancy among trials and treatment types. All control, bentonite clay, and expanding foam burrows were included in this analysis. Proportional data for occupied burrows were normal; therefore, these data were not transformed prior to analyses.

We used a one-way ANOVA to compare the effect of treatment (control, bentonite clay, and expanding foam) on the proportion of burrows occupied by dead crayfish (11 combinations: 4 combinations for control and bentonite clay, 3 combinations for expanding foam). Tukey's HSD post-hoc paired comparisons were used to determine significant differences in mean proportion of burrows occupied by dead crayfish among treatments. Proportional data for burrows occupied by dead crayfish were normal; therefore, these data were not transformed prior to analyses.

Results

Morphometric variables

We collected 60 crayfish (34 female, 26 male) from 47 burrows excavated during both trials (Table 1). Mean CL (mm) of females (38.6 ± 1.0) was the same as male CL (38.6 ± 1.0) (Bates et al. 2023). Mean burrow depth (cm) for occupied burrows (n = 47; 56.1 ± 3.3) was similar to the depth of unoccupied burrows (n = 63; 51.4 ± 2.8) as was mean entrance diameter (mm) for occupied burrows (n = 47; 30.3 ± 2.1) and unoccupied burrows (n = 63; 26.2 ± 1.7) (Bates et al. 2023). Mean carapace length (mm) did not differ between live (n = 6; 36.9 ± 1.5) and dead (n = 13; 41.0 ± 1.9) crayfish

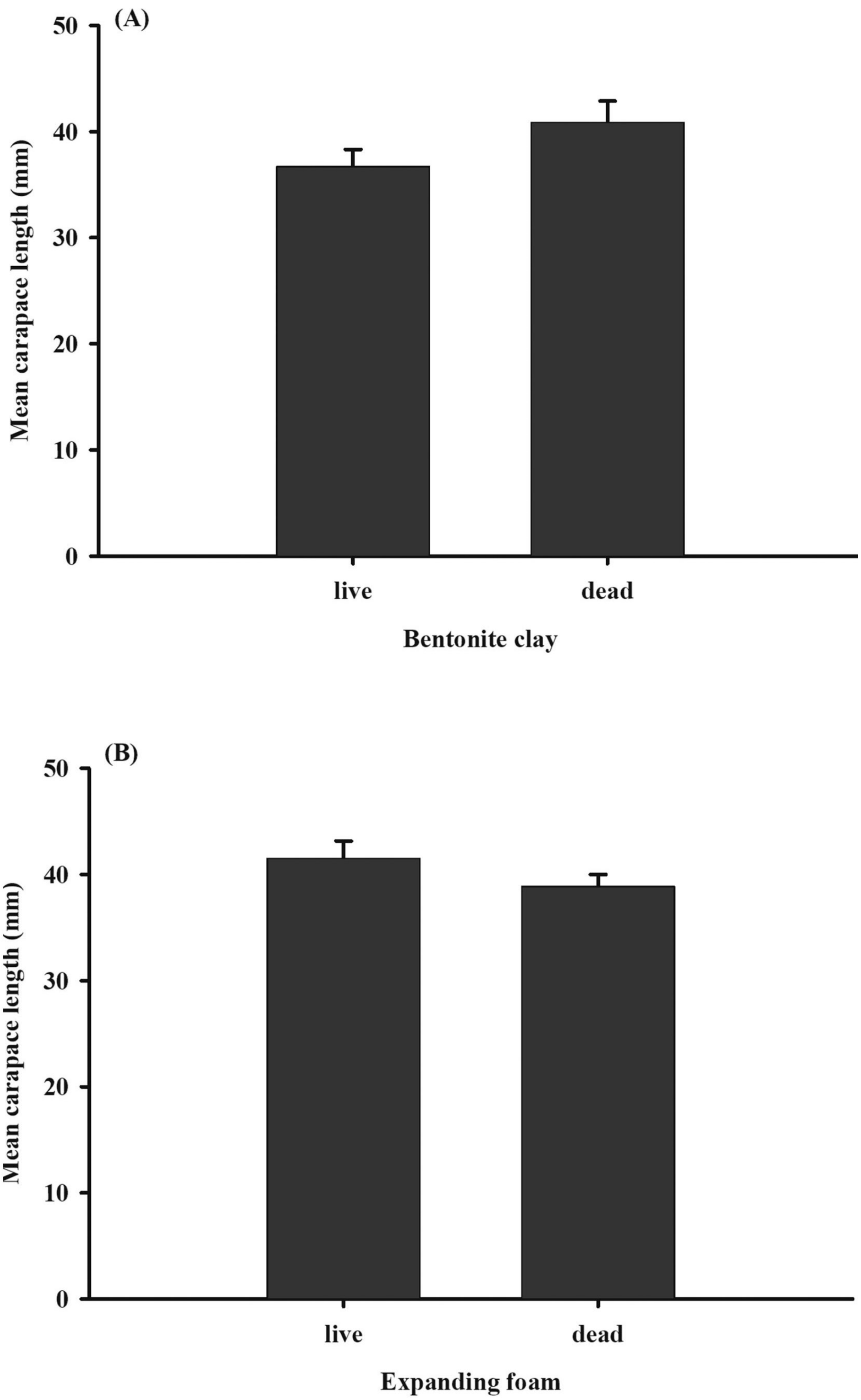

Figure 2. Mean carapace length of live and dead crayfish in occupied treated burrows; trials and ponds combined for (A) bentonite clay (n = 6 live and n = 13 dead) and (B) expanding foam (n = 6 live and n = 12 dead). There was no significant difference in mean carapace length between live and dead crayfish treated with bentonite clay ($p = 0.1$) or expanding foam ($p = 0.2$). Error bars represent ± 1 standard error.

treated with bentonite clay (Mann-Whitney, U = 56.5, $p = 0.1$) (Figure 2A) or between live (n = 6; 41.7 ± 1.5) and dead (n = 12; 39.0 ± 1.0) crayfish treated with expanding foam (t = −1.49, df = 16, $p = 0.2$) (Figure 2B).

Mean depth (cm) of burrows treated with bentonite clay was significantly greater for burrows occupied by live (n = 4; 43.0 ± 4.0) crayfish than by dead

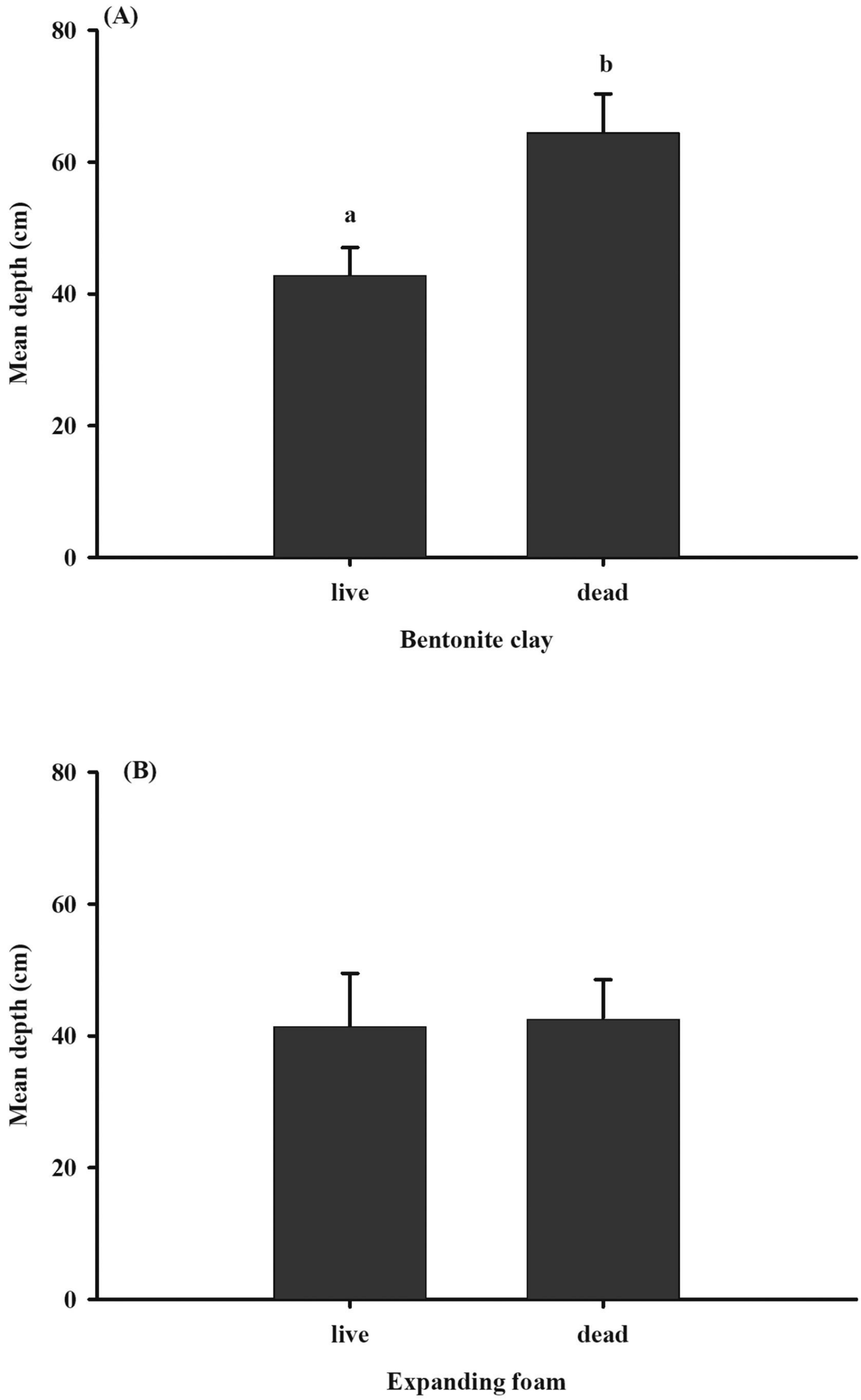

Figure 3. Mean depth of burrows occupied by live or dead crayfish after treatments; trials and ponds combined for (A) bentonite clay (n = 4 live and n = 11 dead) and (B) expanding foam (n = 5 live and n = 8 dead). Letters denote a significant difference in mean depth of burrows occupied by live and dead crayfish for bentonite clay (*p* = 0.05). There was no significant difference in mean depth of burrows occupied by live and dead burrow crayfish for expanding foam (*p* = 0.9). Error bars represent ± 1 standard error.

(n = 11; 64.6 ± 5.7) crayfish (t = 2.16, df = 13, *p* = 0.05) (Figure 3A); however, mean depth of burrows treated with expanding foam was not different between burrows occupied by live (n = 5; 41.6 ± 7.9) or dead (n = 8; 42.8 ± 5.8) crayfish (t = 0.12, df = 11, *p* = 0.9) (Figure 3B). Mean burrow entrance diameter

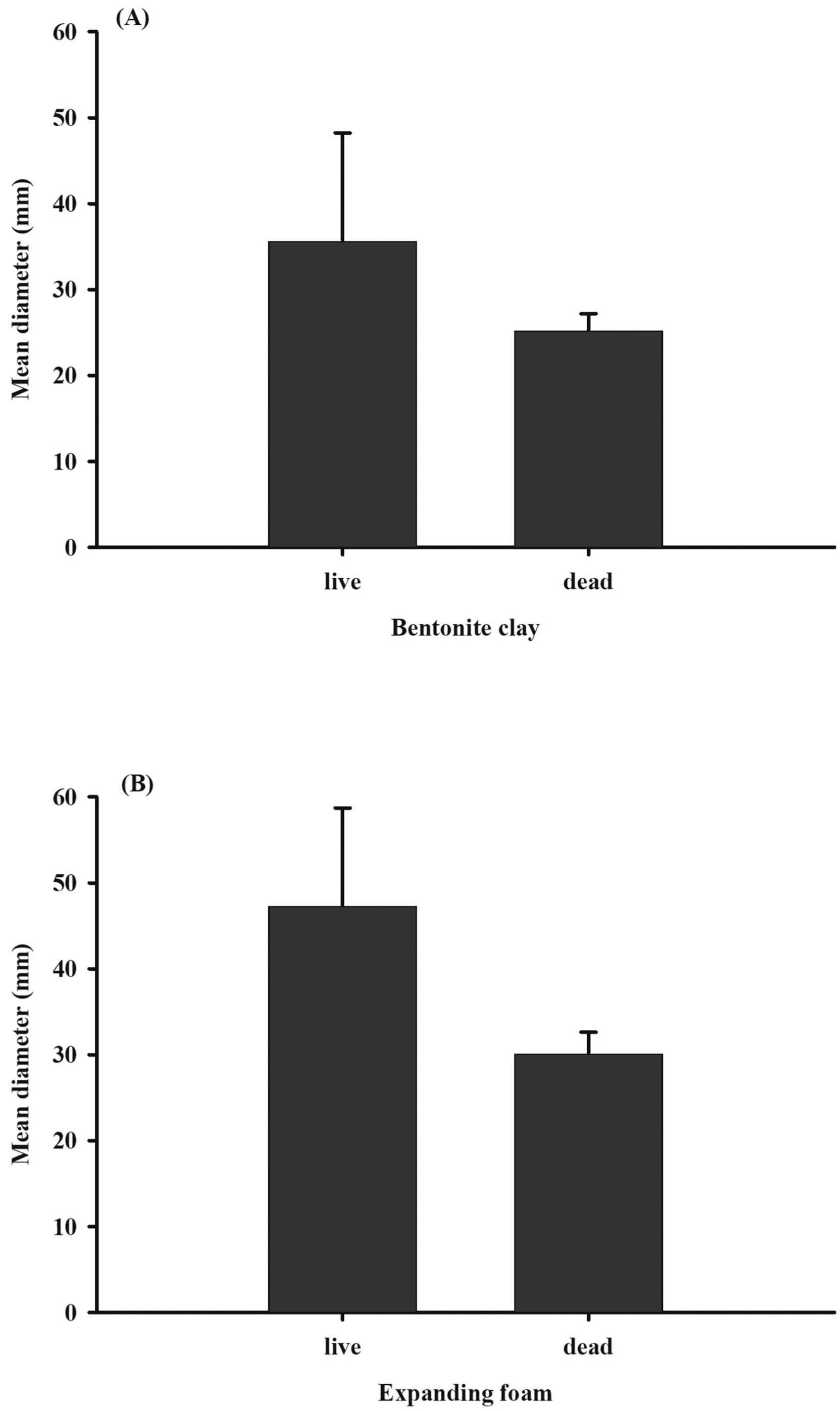

Figure 4. Mean entrance diameter of burrows occupied by live or dead crayfish after treatments; trials and ponds combined for (A) bentonite clay (n = 4 live and n = 11 dead) and (B) expanding foam (n = 5 live and n = 8 dead). There was no significant difference in mean diameter of burrows occupied by live and dead crayfish for bentonite clay (p = 0.8) or expanding foam (p = 0.2). Error bars represent ± 1 standard error.

(mm) treated with bentonite clay was not significantly different for burrows occupied by live (n = 4; 35.8 ± 12.5) or dead (n = 11; 25.4 ± 2.0) crayfish (Mann-Whitney, U = 19.5, p = 0.8) (Figure 4A) or for burrows treated with expanding foam occupied by live (n = 5; 47.4 ± 11.3) or dead (n = 8; 30.3 ± 2.4) crayfish (Mann-Whitney, U = 10.5, p = 0.2) (Figure 4B).

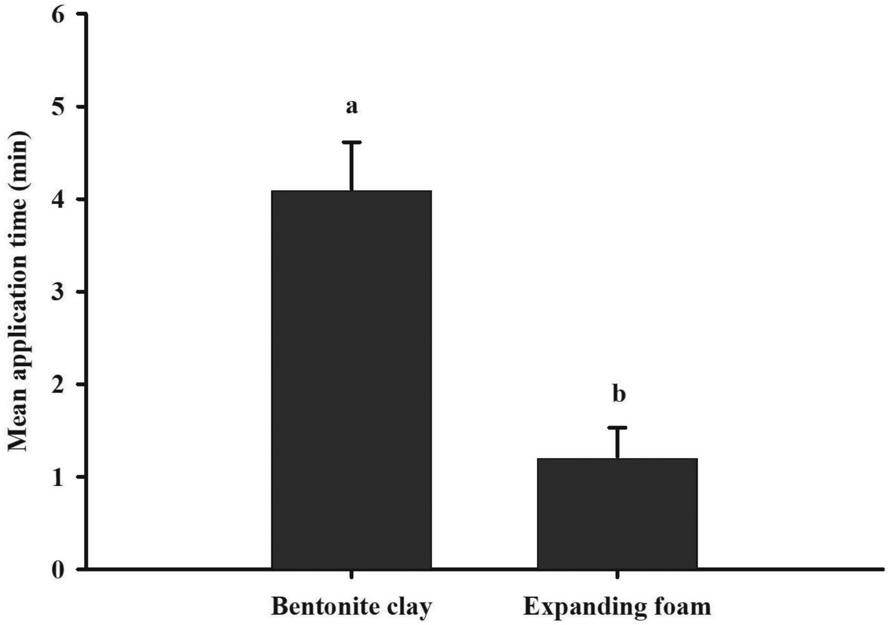

Figure 5. Mean time (min) taken to apply blocker treatments per burrow (i.e., bentonite clay: n = 8 and expanding foam: n = 8). Letters denote a significant difference in application times (p = 0.00003). Error bars represent ±1 standard error.

Mean application time for treatments and mean occupancy of burrows

Mean application time (min) for treating burrows with bentonite clay (n = 8; 4.1 ± 0.5) was significantly greater than for burrows treated with expanding foam (n = 8; 1.2 ± 0.3; t = 4.85, df = 14, p = 0.0003) (Figure 5). There was no significant effect of treatment (control: n = 8, bentonite clay: n = 8, expanding foam: n = 8; $F_{2,18} = 1.40$, $p = 0.3$) (Figure 6A) or trial (Trial 1: n = 12, Trial 2: n = 12; $F_{1,18} = 0.48$, $p = 0.5$) (Figure 6B) on the mean proportion of occupancy, nor was there a statistically significant interaction in occupancy between treatments and trials ($F_{2,18} = 1.71$, $p = 0.2$) (Table 2).

Mean proportion of occupied burrows with dead crayfish per treatment

We estimated proportional mortality for both the control and bentonite clay treatments for each pond during both trials (i.e., control: n = 4 and bentonite clay: n = 4); however, there were no occupied burrows treated with expanding foam in H25 during both trials, which resulted in only three proportional mortality estimates for expanding foam (Table 3). Mean proportion of burrows occupied by dead crayfish was significantly different among treatments (control: n = 4, bentonite clay: n = 4, expanding foam: n = 3; $F_{2,8} = 15.6$, $p = 0.002$) (Table 4; Figure 7A). Proportion of occupied burrows with dead crayfish was significantly greater for bentonite clay treatments (74%) compared to control treatments (6%) ($p = 0.002$), and for expanding foam treatments (62%) compared to control treatments ($p = 0.009$). There was no significant difference in the proportion of occupied burrows with dead crayfish for bentonite clay treatments compared to expanding foam treatments ($p = 0.7$) (Table 5; Figure 7A). If bentonite clay reached groundwater

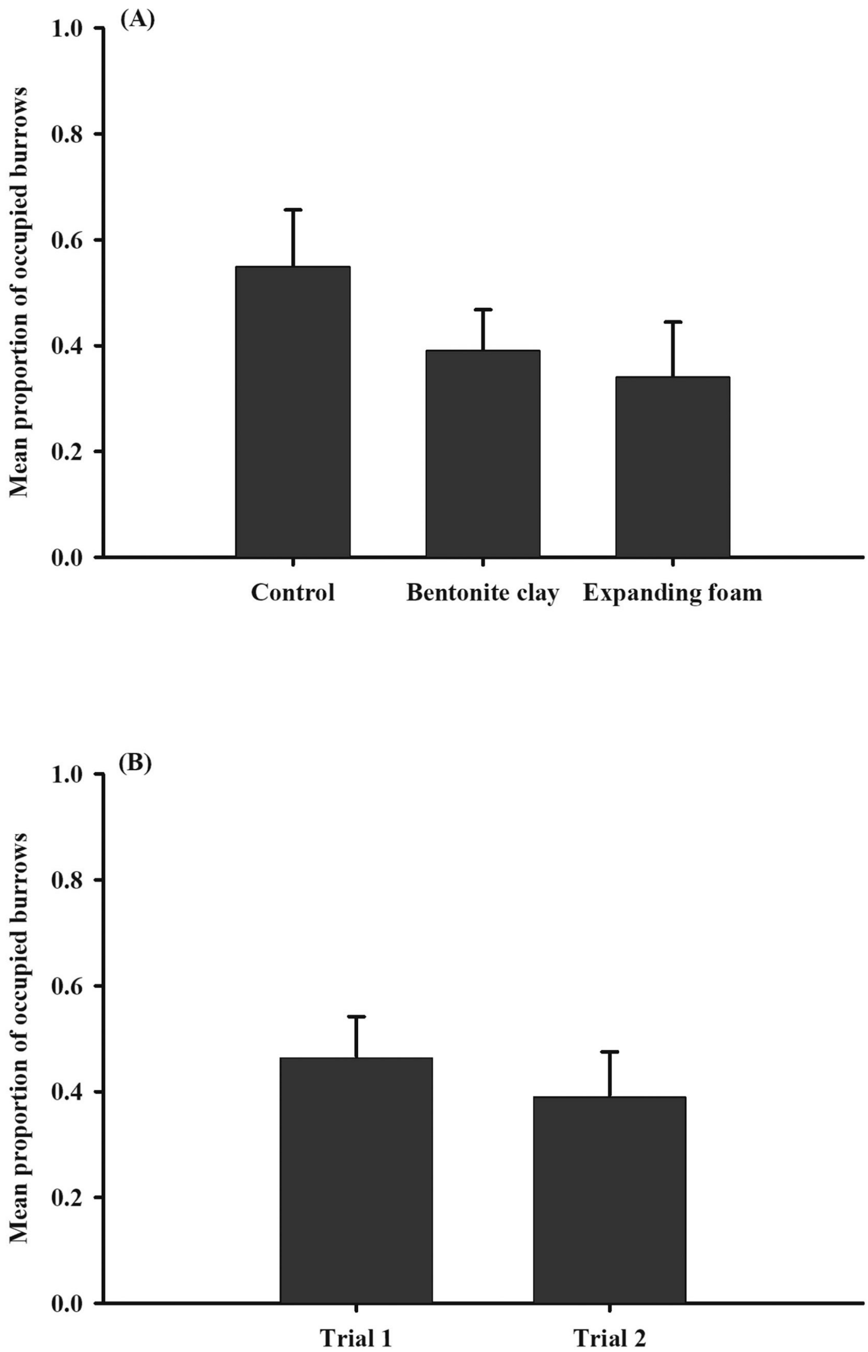

Figure 6. Mean proportion of burrow occupancy within each treatment (bentonite clay: n = 8, expanding foam: n = 8, control: n = 8) and trial (Trial 1: n = 12, Trial 2: n = 12); data for ponds combined. There was no significant difference in the mean proportion of occupied burrows between (A) treatments (p = 0.3) or (B) trials (p = 0.5). Error bars represent ± 1 standard error.

in the terminal chambers, mortality was 100% compared to 43% if it did not reach the groundwater. Similarly, if expanding foam reached the groundwater, mortality was 83% compared to 43% if it did not reach the groundwater in the terminal chamber (Figure 7B).

Discussion

Invasive crayfish are serious pests in aquatic and terrestrial ecosystems because they can alter habitats, negatively impact native crayfish, and cause changes to ecosystem function (Hein et al. 2007; Hobbs and Lodge 2010; Lodge

Table 2. Analysis of variance (ANOVA) results showing the effect of treatment, trial, and two-way interaction on the mean proportion of occupied burrows. There was no significant difference among treatments, between trials or for the interaction of treatment and trial on the proportion of occupied burrows ($p > 0.05$).

	Proportion of occupied burrows				
Source of variation	SS	df	MS	F	*p*
Treatment	0.189	2	0.095	1.40	0.27
Trial	0.033	1	0.033	0.48	0.50
Interaction	0.232	2	0.116	1.71	0.21
Residuals	1.219	18	0.068		
Total	1.672	23	0.311		

Table 3. Number of burrows with live and dead crayfish for each pond, treatment, and trial.

	Trial 1		Trial 2	
Treatment/ pond number	Live	Dead	Live	Dead
Control				
H19	2	0	1	0
H20	2	0	2	0
H24	4	0	4	0
H25	1	0	2	1
Total	9	0	9	1
Bentonite clay				
H19	0	1	1	1
H20	2	1	0	1
H24	1	3	0	1
H25	0	2	0	1
Total	3	7	1	4
Expanding foam				
H19	1	1	0	1
H20	1	3	0	1
H24	2	1	1	1
H25	0	0	0	0
Total	4	5	1	3

Table 4. Analysis of variance (ANOVA) results showing the effect of treatment on the mean proportion of occupied burrows with dead crayfish. Asterisk denotes a significant difference for treatments ($p < 0.05$).

Source of variation	Proportion of occupied burrows with dead crayfish				
	SS	df	MS	F	*p*
Treatment	1.028	2	0.514	15.58	0.002*
Residuals	0.264	8	0.033		
Total	1.292	10	0.547		

et al. 2012; Twardochleb et al. 2013). Negative impacts include the introduction of pathogens such as *Aphanomyces astaci* [Schikora, 1906], commonly known as crayfish plague, which has caused significant harm to native crayfish species in Europe (Diéguez-Uribeondo and Söderhäll 1993; Jussila et al. 2021). Research focused on strategies to control invasive crayfish has focused on open-water populations (Hyatt et al. 2004; Peay et al. 2019); however, terrestrial burrows provide a refuge from physical, chemical, and biological treatments. We investigated a novel approach to controlling invasive crayfish in burrows by applying two physical blockers, bentonite clay and expanding foam, into burrows and assessing the proportion of occupied burrows with dead crayfish. Treatments were largely successful; however, we identified

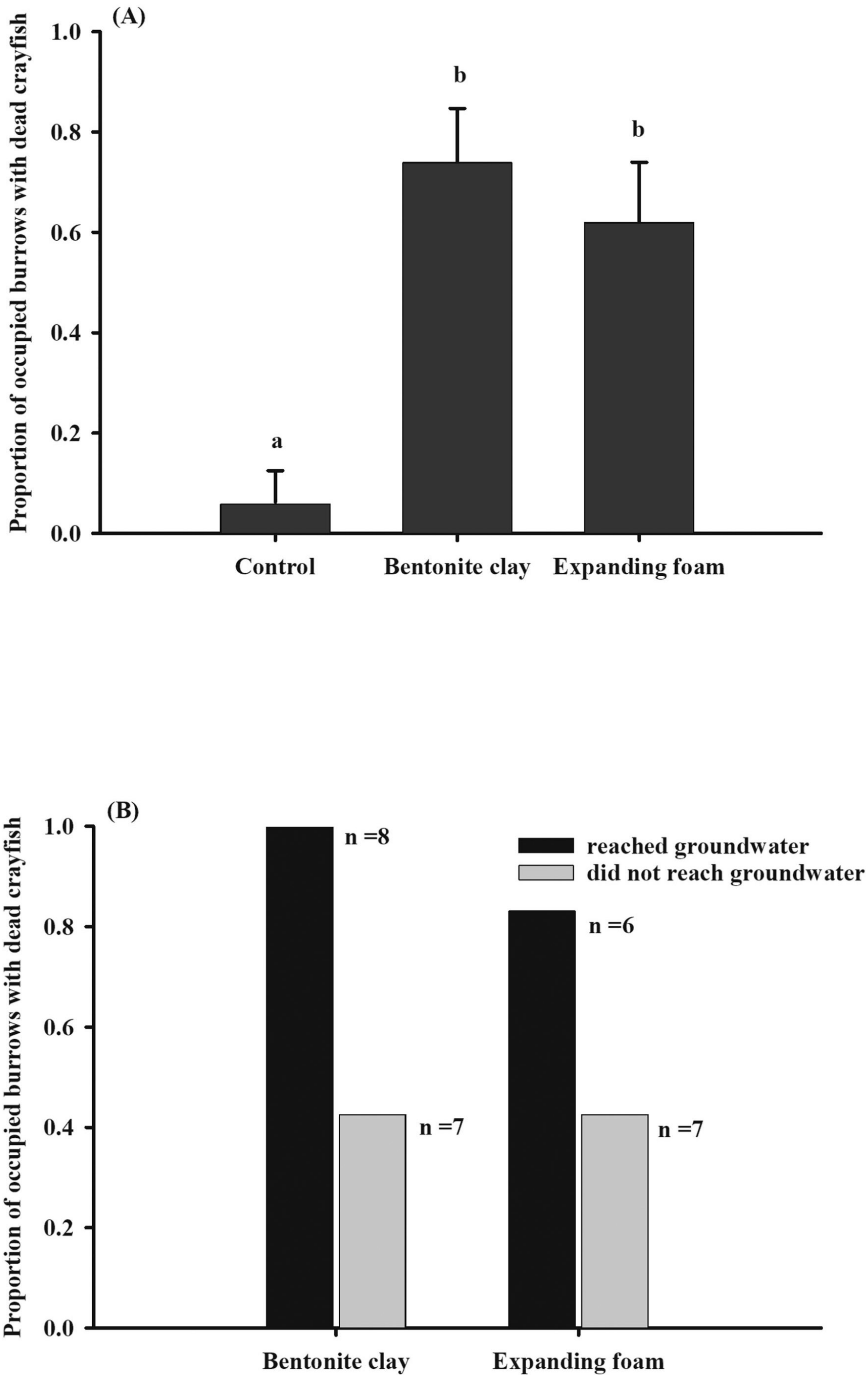

Figure 7. (A) Mean proportion of occupied burrows with dead crayfish for each treatment (i.e., bentonite clay: n = 4, expanding foam: n = 3, control: n = 4). Letters denote a significant difference in mortality between treatments ($p = 0.002$). Error bars represent ± 1 standard error. (B) Proportion of occupied burrows with dead crayfish if treatments did or did not reach the groundwater in the terminal chamber. Only a single proportion was calculated for each bar.

challenges that remain for resource managers as they deploy this approach (tool) in their integrative pest management plans focused on controlling invasive crayfish infestations.

Mortality was significantly greater for both physical blocker treatments than for the control treatment, with mortality greater than 60% in occupied burrows treated with either bentonite clay or expanding foam compared to

Table 5. Tukey's HSD post-hoc multiple comparisons. Asterisk denotes a significant difference among treatments ($p < 0.05$) (i.e., bentonite clay: n = 4, expanding foam: n = 3, control: n = 4).

Treatment	95% confidence interval			
	Mean difference	Lower	Upper	*p* adjusted
Control - bentonite clay	−0.679	−1.046	−0.312	0.002*
Expanding foam - bentonite clay	−0.119	−0.516	0.277	0.678
Expanding foam - control	0.560	0.163	0.956	0.009*

6% in control burrows. A possible cause of crayfish mortality may be the lack of oxygen in treated burrows. Stoeckel et al. (2021) showed that crayfish burrow chimneys can serve as passive ventilation systems, which is an advantageous outcome of construction since burrow water typically contains low oxygen concentrations (< 2 mg L^{-1}); and it has been speculated that crayfish primarily rely on air breathing in burrows rather than by obtaining oxygen from hypoxic or anoxic burrow water (Grow and Merchant 1980; Huner and Barr 1991; Ilhéu et al. 2003; Souty-Grosset et al. 2014). Future studies could evaluate whether water quality of the groundwater in terminal chambers alters the effectiveness of treatments; however, we saw higher mortality in burrows if bentonite clay or expanding foam reached the groundwater in terminal chambers. If the physical blockers reach the groundwater, the air space between the terminal chamber and entrance is eliminated by solid mass of clay or foam. Crayfish do plug their burrows during prolonged periods of high temperature and drought (Huner and Barr 1991; Stoeckel et al. 2021), so gill fouling by bentonite clay or expanding foam may also be a factor causing crayfish mortality. Both physical blockers may impair gill function (e.g., oxygen or ammonia exchange) (Holdich 2002) because they coat crayfish with either a very sticky foam or clay.

The soil in the research ponds used in the experimental trials is composed of a sand/silt/clay mixture which is readily dug by *P. clarkii* (Correia and Ferreira 1995; Ames et al. 2015). Despite the large number of crayfish stocked into each pond (i.e., ~ 800/pond) over five months, we observed relatively few burrows (i.e., ~ 28/pond or 110 total) in September, and of these, only 43% were occupied. Predation may have been a factor affecting the number of burrows and burrow occupancy because *P. clarkii* are an important source of food for mammals and birds (Adrián and Delibes 1987; Delibes and Adrián 1987; Huner and Barr 1991; Beja 1996; Correia 2001), and predation is a common cause of mortality in crayfish (Momot et al. 1978). While no fish inhabited the ponds before or during the study, mammals, primarily common raccoon (*Procyon lotor* [Linnaeus, 1758]) and birds, primarily great blue heron (*Ardea herodias* [Linnaeus,1758]), great egret (*Ardea alba* [Linnaeus,1758]), and black-crowned night heron (*Nycticorax nycticorax* [Linnaeus, 1758]), regularly disturbed (dug) burrows and left tracks and carcasses around ponds. Future studies can potentially prevent high predation rates by covering trial-ponds with protective netting. Vegetation cover may also be critical to protecting crayfish from predation and thus increase burrowing rates in ponds (Huner and Barr 1991; LSU 2007). Rates of burrowing increase with moisture levels in soils; therefore, ponds should be drained slowly (Huner and Barr 1991). The use of soaker hoses to supplement soil moisture may also improve burrowing rates in ponds. Finally, ensuring that only adult crayfish are stocked into ponds may enhance rates of burrowing due to their use of burrows during reproduction (LSU 2007).

The entrances of burrows with dead crayfish were not significantly wider than burrows with live crayfish for either physical blocker treatment. Mean depth (0.53 m) of terminal chambers in our study was within the range of depths (0.28–0.58 m) documented for *P. clarkii* burrows (Correia and Ferreira 1995). We were concerned that bentonite clay and expanding foam would be less effective in deeper and/or narrower burrows; however, this was not the case. There was no difference in the proportion of burrows occupied by dead crayfish based on burrow depth for burrows treated with expanding foam, possibly due to how expanding foam is designed to expand to fill voids which likely increased the chance of it reaching the terminal chamber. There was a statistically significant difference in mortality for burrows treated with bentonite clay; however, crayfish mortality was greater in burrows that were deeper. We believe treatments were more effective if the physical blockers reached the terminal chamber and were most effective when they reach the groundwater in the terminal chamber. Bentonite clay granules can clump together and form a "plug" before reaching the groundwater in terminal chambers; however, the use of PVC pipe "slides" (e.g., 5-cm PVC pipes cut longitudinally in half) to deliver clay particle and water into burrows may reduce the chance of the clay particles clumping to burrow walls and increase the likelihood that clay particles reach the terminal chamber (B. Wright, Michigan Department of Natural Resources, personal communication, July 27, 2022).

Procambarus clarkii typically have a relatively simple burrow morphology compared to other crayfish species that are primary burrowers (Huner and Barr 1991; Correia and Ferreira 1995; Palaoro et al. 2013). Most of the burrows in our study had a simple morphology with only a single entrance and no sharp angles descending to the groundwater found in terminal chambers. It is possible that blockers would be less effective in burrows with complex structure (i.e., multiple entrances, strongly angled tunnels). Additional research to improve application methods, such as the use of compressed air to deliver bentonite clay particles into burrows, would increase the likelihood that physical blockers reach terminal chambers, regardless of burrow structure.

We observed that physical blockers completely seal burrow entrances and no reverse burrows or escape tunnels were noted around treated burrows. Non-target organisms within crayfish burrows would likely be trapped within burrows after treatments are applied. It appears that treatments conducted within one meter of the edges of infested stormwater retention ponds could significantly impact native primary burrowing crayfish; however, native crayfish burrows tend to be located more than one meter from pond edges and are not connected to permanent bodies of water based on field observations (K. Quebedeaux, Michigan Department of Natural Resources, personal communication, June 15, 2023). Burrows of native crayfish also appear to be deeper and more complex than those of *P. clarkii*, which may be protective of native crayfish, since the treatments used in this study tended to be less effective in complex burrows. Native crayfish species that have been sampled at *P. clarkii*-infested sites in Michigan include the paintedhand mudbug (*Lacunicambarus polychromatus* [Thoma, Jezerinac & Simon, 2005]), virile crayfish (*Faxonius virilis* [Hagen, 1870]), and calico crayfish (*Faxonius immunis* [Hagen, 1870]) (B. Wright, Michigan Department of Natural Resources, personal communication, June 15, 2023). Tertiary burrowers such as *F. virilis* rarely burrow, except in time of low water, so they are less likely to be affected by burrow treatments but are not completely exempt from potential nontarget impacts of burrow treatments (Caldwell and Bovbjerg 1969; Bovbjerg 1970).

Graham's crawfish snakes (*Regina grahamii* [Baird & Girard, 1853]) occur in wetlands from the lower Mississippi River floodplain, the Red River Valley, and Upper Mississippi Delta/Ouachita River bottoms and can reside in crayfish burrows and feed almost exclusively on crayfish; their populations are currently considered secure (Boundy and Carr 2017). The massasauga rattlesnake (*Sistrurus catenatus* [Rafinesque-Schmaltz, 1818]) is not known to feed on crayfish but will reside in their burrows during spring (Seigel 1986). Hine's emerald dragonfly (*Somatochlora hineana* [Williamson, 1931]) is a federally endangered species whose range occurs in wetlands and marshes in southern Missouri, Illinois, Wisconsin, Michigan, and Ontario, Canada (Vogt and Cashatt 1994; Monroe and Britten 2014) and has reportedly been observed to inhabit crayfish burrows and feed on other invertebrates found in groundwater in terminal chambers (Pintor and Soluk 2006). Other small organisms like copepods, water fleas, oligochaetes, and wasps may also inhabit crayfish burrows (Huner and Barr 1991; Glon and Thoma 2017). Because small vertebrates and invertebrates are opportunistic and take refuge in abandoned crayfish burrows, local resource managers could consider identifying threatened and endangered animals at infested sites before the application of physical blockers.

The time associated with any control method is a significant concern for resource managers tasked with controlling invasive species. We found that it took an average of 4 min per burrow to apply bentonite clay into burrows. By comparison, it took an average of 1 min per burrow to apply expanding foam. Although expanding foam took less time to apply, the benefits of using bentonite clay to control invasive crayfish in burrows may outweigh the time difference because it is a natural substance while the components of expanding foam are highly mobile in soils, expected to degrade slowly and persist in the environment, and are acutely toxic to aquatic organisms (Great Stuff 2019). It is unlikely that resource managers would need to obtain a permit to treat burrows with bentonite clay, and its use may mitigate existing damage around infested waterbodies caused by burrowing crayfish. Additionally, bentonite clay may be used in combination with other control methods, such as pesticides, to treat crayfish in burrows. Sealing burrow entrances with bentonite clay after pesticide treatments could prevent affected crayfish from emerging from burrows, thereby mitigating the need to return to treated sites to remove dead crayfish and reducing potential nontarget impacts to predators that may feed on pesticide-laden crayfish.

Conclusions

This study suggests that the use of physical blockers has the potential to be effective for the control of invasive crayfish in burrows. The physical blockers caused high mortality in treated burrows, and they are relatively time effective compared to other control methods. Continued field testing and the evaluation of bentonite clay in an adaptive management framework may confirm its use as an effective tool for integrative pest management plans targeting infestations of invasive crayfish.

Author's contribution:

All authors contributed to portions of the study conception and design. Material preparation, data collection and analysis were performed by B. Bates, M. Wildhaber, and J. Stoeckel. The first draft of the manuscript was written by B. Bates, and all authors edited previous versions of the manuscript. All authors read and approved the final manuscript.

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Disclaimer

Any use of trade, firm, or product names is for descriptive purposes only and does not constitute endorsement by the U.S. Government.

Ethics approval

All animal testing protocols were approved by the USGS Columbia Environmental Research Center Animal Care and Use Committee and conformed to AFS/AIFBR/ASIH (2014) guidelines for the use of fish in research.

Availability of data and material

Data generated during this study are available in the USGS ScienceBase repository, (https://doi.org/10.5066/P96V08D0).

References

Abdelrahman HA, Gibson RL, Fogelman KJ, Cupp AR, Allert AL, Stoeckel JA (2021) Evaluation of dissolved carbon dioxide to stimulate emergence of red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) from infested ponds. *Management of Biological Invasions* 12: 952–974, https://doi.org/10. 3391/mbi.2021.12.4.11

Adrián MI, Delibes M (1987) Food habits of the otter (*Lutra lutra*) in two habitats of the Doñana National Park, SW Spain. *Journal of Zoology* 212: 399–406, https://doi.org/10.1111/j.1469-7998.1987.tb02911.x

Ames CW, Helms BS, Stoeckel JA (2015) Habitat mediates the outcome of a cleaning symbiosis for a facultatively burrowing crayfish. *Freshwater Biology* 60: 989–999, https://doi.org/10.1111/fwb.12559

Anastácio PM, Marques JC (1997) Crayfish, *Procambarus clarkii*, effects on initial stages of rice growth in the lower Mondego River valley (Portugal). *Freshwater Crayfish* 11: 608–617, http://hdl.handle.net/10316.2/32694

Anastácio PM, Parente VS, Correia AM (2005) Crayfish effects on seeds and seedlings: identification and quantification of damage. *Freshwater Biology* 50: 697–704, https://doi.org/10.1111/j.1365-2427.2005.01343.x

Barbaresi S, Tricarico E, Gherardi F (2004a) Factors inducing the intense burrowing activity of the red-swamp crayfish, *Procambarus clarkii*, an invasive species. *Naturwissenschaften* 91: 342–345, https://doi.org/10.1007/s00114-004-0533-9

Barbaresi S, Santini G, Tricarico E, Gherardi F (2004b) Ranging behaviour of the invasive crayfish, *Procambarus clarkii* (Girard). *Journal of Natural History* 38: 2821–2832, https://doi.org/10.1080/00222930410001663308

Boundy J, Carr JL (2017) Amphibians and reptiles of Louisiana: an identification and reference guide. LSU Press, Baton Rouge, Louisiana, 386 pp

Bovbjerg RV (1970) Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). *Ecology* 51: 225–236, https://doi.org/10.2307/1933658

Caldwell MJ, Bovbjerg RV (1969) Natural history of the two crayfish of northwestern Iowa, *Orconectes virilis* and *Orconectes immunis*. *Proceedings of the Iowa Academy of Science* 76: 463–472, https://scholarworks.uni.edu/pias/vol76/iss1/62

Cecchinelli E, Aquiloni L, Maltagliati G, Orioli G, Tricarico E, Gherardi F (2012) Use of natural pyrethrum to control the red swamp crayfish *Procambarus clarkii* in a rural district of Italy. *Pest Management Science* 68: 839-844, https://doi.org/10.1002/ps.2335

Chang VC, Lange WH (1967) Laboratory and field evaluation of selected pesticides for control of the red crayfish in California rice fields. *Journal of Economic Entomology* 60: 473–477, https://doi.org/10.1093/jee/60.2.473

Chucholl C (2011) Population ecology of an alien "warm water" crayfish (*Procambarus clarkii*) in a new cold habitat. *Knowledge and Management of Aquatic Ecosystems* 401: 29, https://doi.org/10.1051/kmae/2011053

Correia AM (2001) Seasonal and interspecific evaluation of predation by mammals and birds on the introduced red swamp crayfish *Procambarus clarkii* (Crustacea, Cambaridae) in a freshwater marsh (Portugal). *Journal of Zoology* 255: 533–541, https://doi.org/10.1017/S0952836901001625

Correia AM, Ferreira Ó (1995) Burrowing behavior of the introduced red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) in Portugal. *Journal of Crustacean Biology* 15: 248–257, https://doi.org/10.1163/193724095X00262

Delibes M, Adrián MI (1987) Effects of crayfish introduction on otter *Lutra lutra* food in the Doñana National Park, SW Spain. *Biological Conservation* 42: 153–159, https://doi.org/10.1016/0006-3207(87)90021-8

Diéguez-Uribeondo J, Söderhäll K (1993) *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci* Schikora. *Aquaculture Research* 24: 761–765, https://doi.org/10.1111/j.1365-2109.1993.tb00655.x

Faller M, Harvey GL, Henshaw, AJ, Bertoldi W, Bruno MC, England J (2016) River bank burrowing by invasive crayfish: Spatial distribution, biophysical controls and biogeomorphic significance. *Science of the Total Environment* 569: 1190–1200, https://doi.org/10.1016/j.scitotenv.2016.06.194

Fredricks KT, Tix JA, Smerud JR, Cupp AR (2020) Laboratory trials to evaluate carbon dioxide as a potential behavioral control method for invasive red swamp (*Procambarus clarkii*) and rusty crayfish (*Faxonius rusticus*). *Management of Biological Invasions* 11: 259, https://doi.org/10.3391/mbi.2020.11.2.06

Gherardi F, Barbaresi S (2000) Invasive crayfish: Activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archiv für Hydrobiologie* 150: 153–168, https://doi.org/10.1127/archiv-hydrobiol/150/2000/153

Gherardi F, Barbaresi S, Salvi G (2000) Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences* 62: 179–193, https://doi.org/10.1007/PL00001330

Gherardi F, Tricarico E, Ilhéu M (2002) Movement patterns of an invasive crayfish, *Procambarus clarkii*, in a temporary stream of southern Portugal. *Ethology Ecology & Evolution* 14: 183–197, https://doi.org/10.1080/08927014.2002.9522739

Glon MG, Thoma RF (2017) An observation of the use of devil crayfish (*Cambarus* cf. *diogenes*) burrows as brooding habitat by Eastern cicada killer wasps (*Sphecius speciosus*). *Freshwater Crayfish* 23: 55–57, https://doi.org/10.5869/fc.2017.v23-1.55

Grow L, Merchant H (1980) The burrow habitat of the crayfish, *Cambarus diogenes diogenes* (Girard). *American Midland Naturalist* 103: 231–237, https://doi.org/10.2307/2424621

Haubrock PJ, Inghilesi AF, Mazza G, Bendoni M, Solari L, Tricarico E (2019) Burrowing activity of *Procambarus clarkii* on levees: analysing behaviour and burrow structure. *Wetlands Ecology and Management* 27: 497–511, https://doi.org/10.1007/s11273-019-09674-3

Hein CL, Vander Zanden MJ, Magnuson JJ (2007) Intensive trapping and increased fish predation cause massive population decline of an invasive crayfish. *Freshwater Biology* 52: 1134–1146, https://doi.org/10.1111/j.1365-2427.2007.01741.x

Hobbs HH, Lodge DM (2010) Decapoda. In: Thorp JH, Covich AP (eds), Ecology and Classification of North American Freshwater Invertebrates (Third Edition). Academic Press, San Diego, pp 901–967, http://dx.doi.org/10.1016/B978-0-12-374855-3.00028-5

Hobbs HH, Jass JP, Huner JV (1989) A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana* 56: 299–316, https://www.jstor.org/stable/20104461

Holdich DM (ed) (2002) Biology of freshwater crayfish. Oxford, UK, 702 pp

Huner JV, Barr JE (1991) Red swamp crayfish: Biology and exploitation. The Louisiana Sea Grant College Program, Center for Wetland Resources. Louisiana State University, Baton Rouge, LA, 128 pp

Huner JV, Lindqvist OV (1995) Physiological adaptations of freshwater crayfishes that permit successful aquacultural enterprises. *American Zoologist* 35: 12–19, https://doi.org/10.1093/icb/35.1.12

Hyatt MW (2004) Investigation of crayfish control technology. Final Report Cooperative Agreement no. 1448-20181-02-J850. Arizona Game and Fish Department, Phoenix, Arizona, USA

Ilhéu M, Acquistapace P, Benvenuto C, Gherardi F (2003) Shelter use of the Red Swamp Crayfish (*Procambarus clarkii*) in dry-season stream pools. *Archiv für Hydrobiologie* 157: 535–546, https://doi.org/10.1127/0003-9136/2003/0157-0535

Jin S, Jacquin L, Xiong M, Li R, Lek S, Li W, Zhang T (2019) Reproductive pattern and population dynamics of commercial red swamp crayfish (*Procambarus clarkii*) from China: implications for sustainable aquaculture management. *PeerJ* 7: e6214, https://doi.org/10.7717/peerj.6214

Jurcak AM, Lahman SE, Wofford SJ, Moore PA (2016) Behavior of crayfish. In: Longshaw M, Stebbing P (eds), Biology and ecology of crayfish. CRC Press, Boca Raton, FL, pp 117–131, https://www.taylorfrancis.com/chapters/edit/10.1201/b20073-7/behavior-cray%EF%AC%81-sh-ana-jurcak-sara-lahman-sarah-wo%EF%AC%80-ord-paul-moore

Jussila J, Edsman L, Maguire I, Diéguez-Uribeondo J, Theissinger K (2021) Money kills native ecosystems: European crayfish as an example. *Frontiers in Ecology and Evolution* 9: 1–22, https://doi.org/10.3389/fevo.2021.648495

Lodge DM, Deines A, Gherardi F, Yeo DCJ, Arcella T, Baldridge AK, Barnes MA, Chadderton WL, Feder JL, Gantz CA, Howard GW, Jerde CL, Peters BW, Peters JA, Sargent LW, Turner CR, Wittmann ME, Zeng Y (2012) Global introductions of crayfishes: evaluating the impact of species invasions on ecosystem services. *Annual Review of Ecology, Evolution, and Systematics* 43: 449–472, https://doi.org/10.1146/annurev-ecolsys-111511-103919

Loureiro TG, Anastácio PM, Araujo PB, Souty-Grosset C, Almerão MP (2015) Red swamp crayfish: biology, ecology and invasion-an overview. *Nauplius* 23: 1–19, https://doi.org/10.1590/S0104-64972014002214

Momot WT, Gowing H, Jones PD (1978) The dynamics of crayfish and their role in ecosystems. *American Midland Naturalist* 99: 10–35, https://doi.org/10.2307/2424930

Monroe EM, Britten HB (2014) Conservation in Hine's sight: the conservation genetics of the federally endangered Hine's emerald dragonfly, *Somatochlora hineana*. *Journal of Insect Conservation* 18: 353–363, https://doi.org/10.1007/s10841-014-9643-7

Oficialdegui FJ, Sánchez MI, Clavero M (2020) One century away from home: how the red swamp crayfish took over the world. *Reviews in Fish Biology and Fisheries* 30: 121–135, https://doi.org/10.1007/s11160-020-09594-z
Oluoch AO (1990) Breeding biology of the Louisiana red swamp crayfish *Procambarus clarkii* Girard in Lake Naivasha, Kenya. *Hydrobiologia* 208: 85–92, https://doi.org/10.1007/BF00008447
Palaoro AV, Dalosto MM, Coutinho C, Santos S (2013) Assessing the importance of burrows through behavioral observations of *Parastacus brasiliensis*, a Neotropical burrowing crayfish (Crustacea), in laboratory conditions. *Zoological Studies* 52: 1–8, https://doi.org/10.1186/1810-522X-52-4
Peay S, Johnsen SI, Bean CW, Dunn AM, Sandodden R, Edsman L (2019) Biocide treatment of invasive signal crayfish: successes, failures and lessons learned. *Diversity* 11: 29, https://doi.org/10.3390/d11030029
Peruzza L, Piazza F, Manfrin C, Bonzi LC, Battistella S, Giulianini PG (2015) Reproductive plasticity of a *Procambarus clarkii* population living 10 C below its thermal optimum. *Aquatic Invasions* 10: 199–208, https://dx.doi.org/10.3391/ai.2015.10.2.08
Piersanti S, Pallottini M, Salerno G, Goretti E, Elia AC, Dörr AJM, Rebora M (2018) Resistance to dehydration and positive hygrotaxis in the invasive red swamp crayfish *Procambarus clarkii*. *Knowledge & Management of Aquatic Ecosystems* 419: 36, https://doi.org/10.1051/kmae/2018024
Pintor LM, Soluk DA (2006) Evaluating the non-consumptive, positive effects of a predator in the persistence of an endangered species. *Biological Conservation* 130: 584–591, https://doi.org/10.1016/j.biocon.2006.01.021
R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Reynolds J, Souty-Grosset C, Richardson A (2013) Ecological roles of crayfish in freshwater and terrestrial habitats. *Freshwater Crayfish* 19: 197–218, https://doi.org/10.5869/fc.2013.v19-2.197
Seigel RA (1986) Ecology and conservation of an endangered rattlesnake, *Sistrurus catenatus*, in Missouri, USA. *Biological Conservation* 35: 333–346, https://doi.org/10.1016/0006-3207(86)90093-5
Siesa ME, Manenti R, Padoa-Schioppa E, De Bernardi F, Ficetola GF (2011) Spatial autocorrelation and the analysis of invasion processes from distribution data: a study with the crayfish *Procambarus clarkii*. *Biological Invasions* 13: 2147–2160, https://doi.org/10.1007/s10530-011-0032-9
Souty-Grosset C, Reynolds J, Gherardi F, Aquiloni L, Coignet A, Pinet F, Mancha Cisneros MDM (2014) Burrowing activity of the invasive red swamp crayfish, *Procambarus clarkii*, in fishponds of La Brenne (France). *Ethology Ecology & Evolution* 26: 263–276, https://doi.org/10.1080/03949370.2014.892538
Stoeckel JA, Szoka M, Abdelrahman HA, Davis JD, Blersch DM, Helms BS (2021) *Crayfish chimneys* function as burrow-ventilation structures. *Journal of Crustacean Biology* 41, https://doi.org/10.1093/jcbiol/ruab045
Thomas JR, Masefield S, Hunt R, Wood MJ, Hart AG, Hallam J, Hallam J, Cable J (2019) Terrestrial emigration behaviour of two invasive crayfish species. *Behavioural Processes* 167: 103917, https://doi.org/10.1016/j.beproc.2019.103917
Twardochleb LA, Olden JD, Larson ER (2013) A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science* 32: 1367–1382, https://doi.org/10.1899/12-203.1
Vogt TE, Cashatt ED (1994) Distribution, habitat, and field biology of *Somatochlora hineana* (Odonata: Corduliidae). *Annals of the Entomological Society of America* 87: 599–603, https://doi.org/10.1093/aesa/87.5.599

Web sites and online databases

Bates BL, Wildhaber ML, Stoeckel JA, Allert AL, Elliott GJ, Pieper EB, Fogelman KJ, Gullatte JM, Irwin JC (2023) Crayfish morphometric measurements, burrow attributes and occupancy in response to physical burrow barriers: U.S. Geological Survey data release, https://doi.org/10.5066/P96V08D0
Benseal (2017) Safety data sheet for bentonite clay. https://www.airproductssupply.com/PDF/msdsBENSEAL.pdf (accessed July21, 2023)
Great Stuff (2019) Safety data sheet for Great Stuff. https://labelsds.com/images/user_uploads/Great%20Stuff%20Gaps-Cracks%20SDS%206-29-20.pdf (accessed 22 June 2023)
LSU (2007) Louisiana Agriculture Center Research, Louisiana Agricultural Experiment Station, Louisiana Cooperative Extension Service. Louisiana crawfish production manual, Agriculture Center publication number 2637, chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.lsuagcenter.com/~/media/system/b/4/6/c/b46ccc1439d0d661d9d96267b84db4b7/crawfish%20production%20manualpdf.pdf (accessed 23 June 2023)